US007479573B2

(12) **United States Patent**
Chu et al.

(10) **Patent No.: US 7,479,573 B2**
(45) **Date of Patent: Jan. 20, 2009**

(54) **TRANSFECTION REAGENTS**

(75) Inventors: **Yongliang Chu**, Rockville, MD (US); **Malek Masoud**, Gaithersburg, MD (US); **Gulilat Gebeyehu**, Potomac, MD (US)

(73) Assignee: **Invitrogen Corporation**, Carlsbad, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 281 days.

(21) Appl. No.: **11/040,687**

(22) Filed: **Jan. 21, 2005**

(65) **Prior Publication Data**

US 2005/0164391 A1 Jul. 28, 2005

Related U.S. Application Data

(63) Continuation of application No. 09/438,365, filed on Nov. 12, 1999, now Pat. No. 7,166,745.

(60) Provisional application No. 60/108,117, filed on Nov. 12, 1998.

(51) **Int. Cl.**

C07C 211/62	(2006.01)
C07C 211/63	(2006.01)
C07C 211/64	(2006.01)
A61K 31/13	(2006.01)
A61K 9/127	(2006.01)
A61K 9/50	(2006.01)
C12N 15/88	(2006.01)

(52) **U.S. Cl.** **564/292**; 564/295; 564/286; 514/579; 514/642; 514/643; 435/458; 424/450; 424/499

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,654,785	A	10/1953	Miescher et al.	260/567.6
2,695,314	A	11/1954	Kosmin	260/584
2,867,665	A	1/1959	Dornfield	
2,901,461	A	8/1959	Auerbach et al.	260/47
3,152,188	A	10/1964	Kirkpatrick	260/584
3,324,182	A	6/1967	De Brunner et al.	260/583
3,369,905	A	2/1968	Jones et al.	96/107
4,143,003	A	3/1979	Haas et al.	
4,235,871	A	11/1980	Papahadjopoulos et al.	

(Continued)

FOREIGN PATENT DOCUMENTS

AU	158967	2/1952

(Continued)

OTHER PUBLICATIONS

Banerjee et al., (2001) "Design, Synthesis, and Transfection Biology of Novel Cationic Glycolipids for Use in Liposomal Gene Delivery," J. Med. Chem., 44:4176-4185.

Asokan, A. and Cho, M.J. (2004), "Cytosolic Delivery of Macromolecules. 3. Synthesis and Characterization of Acid-Sensitive Bis-Detergents," Bioconj. Chem. 15:1166-1173.

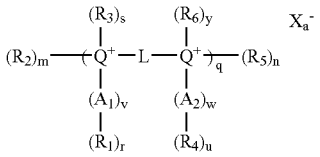
Banerjee, R. et al. (1999), "Novel Series of Non-Glycerol-Based Cationic Transfection Lipids for Use in Liposomal Gene Delivery," J. Med. Chem. 42:4292-4299.

(Continued)

Primary Examiner—Brian J Davis
(74) *Attorney, Agent, or Firm*—Invitrogen Corporation; Emanuel J. Vacchiano; James K. Blodgett

(57) **ABSTRACT**

Disclosed are compounds capable of facilitating transport of biologically active agents or substances into cells having the general structure:



wherein

Q is selected from the group consisting of N, O and S; L is any bivalent organic radical capable of linking each Q, such as C, CH, $(CH_2)l$, or $\{(CH_2)i—Y—(CH_2)j\}k$, wherein Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by $—X_1—L'—X_2—Z$ or $—Z$; $R_1—R_6$, independently of one another, are selected from the group consisting of H, $—(CH_2)$ p-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and wherein at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group; and anyone of R_1, R_3, R_4 and/or R_6 may optionally be covalently linked with each other, with Y or with L when L is C or CH to form a cyclic moiety; Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein; X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene, and arylene; L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether; D is Q or a bond; A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C{NH), C(S) and $(CH_2)t$; X is a physiologically acceptable anion; m, n, r, s, u, v, w and y are 0 or 1, with the proviso that when both m and n are 0 at least one of r, s, u and y is other than 0; i, j, k, l, p and are integers from 0 to about 100; q is an integer from 1 to about 1000; and a is the number of positive charge divided by the valence of the anion.

56 Claims, 4 Drawing Sheets